Kinetic Parameters and Plasma Zinc Concentration Correlate Well with Net Loss and Gain of Zinc from Men[1–3]

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ABSTRACT The search for a reliable, convenient indicator of Zn status was the focus of research for several decades. Plasma Zn concentration is still the most widely used clinical measurement, despite the known problems of interpretation. More recently, researchers suggested that isotopically determined kinetic parameters, such as the exchangeable Zn pool (EZP), may more accurately and reliably reflect body Zn status. The objective of this study was to examine the relationship between net body Zn loss and gain during acute changes in dietary Zn intake with biochemical and kinetic indices of Zn status. Five men participated in an 85-d Zn depletion/repletion study. Net body Zn loss and gain were determined from the difference between dietary plus intravenously administered Zn and Zn excretion. Biochemical indicators of Zn status included plasma Zn, plasma alkaline phosphatase activity, and plasma retinol binding protein concentration. Following intravenous administration of ^{70}Zn or ^{67}Zn, a compartmental model was used to determine EZP mass, fractional Zn absorption, endogenous zinc excretion (EZE), and plasma Zn flux. The changes in total body zinc correlated best with changes in plasma Zn ($r^2 = 0.826$, $P < 0.001$), EZE ($r^2 = 0.773$, $P < 0.001$), and plasma Zn flux ($r^2 = 0.766$, $P < 0.001$). This study confirms that plasma Zn concentration is a valid indicator of whole-body Zn status in the absence of confounding factors; however, further research is needed to determine how kinetic parameters respond to conditions where plasma Zn concentration is known to be unreliable. J. Nutr. 134: 2178–2181, 2004.

KEY WORDS: • *zinc status* • *biochemical indices* • *stable isotopes* • *kinetic analysis* • *compartmental model*

The role of Zn as an essential nutrient in the human diet has been well established and its biochemical roles are many and diverse. The search for a reliable, convenient indicator of Zn status has been the focus of research for several decades. The ideal indicator is one that responds in a unique and predictable way to a net loss of Zn from the body. Plasma or serum Zn concentration is the most widely used measure of Zn status, but the reliability of this index has been criticized because plasma (or serum) Zn concentrations can fall in response to factors unrelated to body Zn loss (1). Zinc-dependent enzymes, in particular alkaline phosphatase (AP),[5] have been reported to respond to changes in dietary Zn intake (2). Zinc is required for the hepatic synthesis of retinol binding protein (RBP), which is responsible for the inter- and intracellular transport of vitamin A. Some cross-sectional studies in humans suggest that low plasma RBP concentrations are associated with suboptimal status (3,4), although this is not a consistent finding (5). Hair Zn concentration has been proposed as a useful index of Zn status in children (6). A correlation between hair and circulating Zn levels was reported following a study in Panamanian children (7), although it can be argued that because hair growth slows down during Zn deficiency, it can only give a historical reflection of status over a long period of time and does not reflect the current situation.

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[2] Some data previously presented in King, J. C., Shames, D. M., Lowe, N. M., Woodhouse, L. R., Sutherland, B., Abrams, S. A., Turnlund, J. R. & Jackson, M. J. (2001) Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men. Am. J. Clin. Nutr. 74: 116–124.

[3] A figure showing the model used to derive the kinetic parameters presented in this paper is available as supplemental data with the online posting of this paper at www.nutrition.org.

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[5] Abbreviations used: AP, alkaline phosphatase; EZE, endogenous zinc excretion; EZP, exchangeable zinc pool; FZA, fractional zinc absorption; MP, metabolic period; MP1, baseline period; MP2, depletion period; MP3, repletion period; RBP, retinol binding protein; SMAC, sequential multiple analysis chemistry; WHNRC, Western Human Nutrition Research Center.

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There are ~2.5–3 g of Zn in the human body, over half of which is present in skeletal muscle (8), but this Zn is not readily released under conditions of deficiency. It is hypothesized that there is a functional pool of Zn within the body that represents <10% of total body Zn. The Zn in this pool has a relatively rapid turnover rate, and the onset of biochemical consequences observed in Zn deficiency are associated with a reduction in the size of this exchangeable Zn pool (EZP) (1). Researchers suggested that a measure of the EZP provides a measure of the Zn available for biochemical functions. Isotopic tracers can be used to determine the mass of the EZP and other potentially useful parameters such as fractional Zn absorption (FZA) and plasma Zn flux (9).

A metabolic study of Zn homeostasis in adult men was conducted at the USDA Western Human Nutrition Research Center (WHNRC). This study provided a unique opportunity to examine the relation between net body Zn loss and gain during acute changes in dietary Zn intake with functional biochemical indices of Zn status and measurements of the EZP, FZA, and plasma Zn flux. One of the purposes of this study was to determine which of these putative indicators of Zn status best reflects the changes in total body Zn during acute, severe dietary Zn depletion and repletion.

SUBJECTS AND METHODS

Subjects. Twelve male subjects, aged 20 to 35 y, were recruited for the study. Five men completed the 3-month protocol for reasons reported previously (10). The data reported in this paper are from these 5 subjects; 3 were Caucasian, 1 was Hispanic, and 1 was Caucasian-Asian. Their mean age was 28 ± 6 y. All subjects were nonsmokers and judged to be healthy on the basis of a routine blood screening, medical history, physical examination, and psychological profile.

Study design. The study protocol was approved by the University of California, Berkeley, Committee for the Protection of Human Subjects, University of California, San Francisco, Committee on Human Research, and the U.S. Department of Agriculture/Agriculture Research Service, Human Studies Review Committee. All subjects gave written, informed consent.

The subjects were housed in a metabolic ward at the WHNRC for 85 d. The study was divided into 3 metabolic periods (MP): a 16-d baseline period (MP1) in which 12.2 mg of Zn was provided daily, a 41-d depletion period (MP2) in which 0.23 mg Zn/d was provided, and a 29-d repletion period (MP3). During MP3 the men were divided into 2 groups. Group A (subjects 2, 3, and 4) was repleted by intravenous administration of 2 overnight infusions of 66 mg of Zn on days 1 and 12 of the repletion period. Thereafter they received the repletion diet containing 12.2 mg Zn/d. Group B (subjects 10 and 11) was repleted by dietary means alone; they were given a diet containing 12.2 mg Zn/d from day 1 of the repletion period.

The diet, which has been described previously in detail (10), was an egg albumen–based, semipurified formula, adequate in all nutrients except Zn, Fe, and Cu. The basic formula provided 761 kJ per day, with 10% of the energy from protein, 60% from carbohydrate, and 30% from fat. The total energy provided for each subject was designed to maintain a constant body weight and ranged from 155 to 192 $kJ \cdot kg^{-1} \cdot d^{-1}$. The daily Zn intake from the basic formula and all the foods fed during the study, based on the analysis of composite diets, was 0.23 ± 0.07 mg/d. Zinc, as a solution of $ZnSO_4$, was added to the formula diet during the baseline period and during the repletion period (except for the 3 subjects who were initially repleted by intravenous Zn administration), to provide a total intake of 12.2 mg Zn/d.

Stable isotope studies. Stable isotopes of Zn, ^{67}Zn (enriched to 90.09% abundance), and ^{70}Zn (enriched to 85.03% abundance) were purchased as Zn oxide from Oakridge National Laboratory. The isotopes were prepared for intravenous administration (9) and infused in the middle of MP1 (1.579 ± 0.101 mg ^{67}Zn), at the end of MP2 (0.299 ± 0.021 mg ^{70}Zn), and at the end of MP3 (0.412 ± 0.006 mg ^{70}Zn). Blood samples were taken at defined time intervals post isotope infusion via a catheter placed in the arm opposite the site of isotope infusion, as described previously (10).

Sample collection and analysis. Complete 24-h urine and stool collections were made for the duration of the study. Blood samples were taken weekly for plasma Zn concentration determination, complete blood count (System 9000, Serono Diagnostics), and sequential multiple analysis chemistry (SMAC)[6] (SmithKline Beecham, Clinical Laboratories). Plasma samples for kinetic modeling were collected as previously reported (10). Precautions against environmental Zn contamination were taken for all diet, blood, and excreta collections and analysis.

The total Zn concentration of the plasma, urine, and feces was determined by atomic absorption spectroscopy (Smith-Hieftje-22; Thermo Jarrell Ash) (9). Urinary Zn excretion during baseline, depletion, and repletion was determined from the mean of six 24-h urine collections. The plasma RBP concentration was determined by radial immunodiffusion (LC-Partigen RBP kit, Behring Diagnostics).

The ratios of Zn stable isotopes in plasma and urine samples at baseline and during repletion periods were determined by inductively coupled plasma mass spectrometry (Sciex ELAN 500 ICP-MS, Perkin-Elmer) (9,10). During the depletion period, where plasma Zn concentration was very low, isotope enrichment was determined by magnetic sector thermal ionization mass spectrometry (Finnigan MAT 261) (10). Isotope enrichment was expressed as the tracer-tracee ratio (9).

Kinetic analysis. A compartmental model was used to analyze the Zn tracer and steady-state mass data (supplemental Fig. 1, available online). The EZP, plasma flux, FZA, and endogenous zinc excretion (EZE) were determined as previously described (10).

Total body Zn loss and gain. The net amount of Zn lost or gained by the body during the depletion and repletion periods was determined from the difference between Zn output (urine and fecal excretion data) and Zn intake (dietary intakes and intravenous infusions of Zn). Integumental losses (~0.3 mg/d under baseline conditions) (11) were not included and were assumed to be within the error of these analyses.

Statistics. Data were tested using repeated-measures ANOVA. When ANOVA indicated significant differences ($P < 0.05$), Tukey's standardized range test with a procedurewise error rate of 5% was used as a follow-up test for pairwise comparisons among the means. Correlation analysis was used to examine the relation between parameters (Microsoft Excel 2000). Values presented are means ± SD.

RESULTS

Clinical observations. During wk 5 of the depletion period (MP2), subject 2 developed lesions around the mouth and nose with a concurrent severe drop in plasma Zn concentration. He also complained of lethargy and general malaise. The depletion period was therefore ended on d 33 of MP2 by administration of an overnight infusion of Zn (66 mg). Within 2 d of the Zn infusion, the subject reported feeling better and within 1 wk the dermatitis of the face had resolved. During wk 6 of MP2, subject 3 developed mucositis and dermatitis of the scrotum, sufficient to cause discomfort while walking. The depletion period of this subject was therefore ended on d 40 of MP2, also by an overnight infusion of 66 mg of Zn. All of the subjects developed 1 or more clinical manifestations of Zn depletion. In general, the symptoms were characterized by dermatitis or inflammation of the mucosal membranes, all of which were rapidly reversed with Zn repletion.

[6] SMAC analyses, glucose, creatinine, blood urea nitrogen/creatinine ratio, sodium, potassium, chloride, calcium, phosphorus, iron, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, triglycerides, total bilirubin, alkaline phosphatase, γ-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, transferrin saturation, uric acid, blood urea nitrogen, magnesium, HDL-cholesterol, LDL-cholesterol, total-/HDL-cholesterol ratio.

TABLE 1

Biochemical and kinetic parameters measured in men during baseline, Zn depletion, and Zn repletion periods[1,2]

Parameter	Baseline (MP 1)	Depletion (MP 2)	Repletion (MP 3)	ANOVA *P* value	*r*	r^2
Plasma Zn, *μmol/L*	11.6 ± 1.6[a]	2.9 ± 1.0[b]	11.6 ± 1.2[a]	0.0001	0.909	0.826
EZE, *μmol/d*	41.3 ± 3.1[a]	4.6 ± 0.4[b]	53.5 ± 21.4[a]	0.0002	0.879	0.773
Plasma Zn flux, *mmol/d*	7.27 ± 0.55[a]	3.53 ± 1.4[b]	7.31 ± 2.20[a]	0.0013	0.881	0.766
FZA	0.26 ± 0.02[b]	1 ± ND[a3]	0.33 ± 0.12[b]	0.0001	−0.870	0.755
Plasma alkaline phosphatase, *U/L*	48.0 ± 13.9[b]	26.2 ± 4.8[c]	69.2 ± 9.6[a]	0.0002	0.841	0.708
Urinary Zn excretion, *μmol/24 h*	7.0 ± 0.2[a]	0.2 ± 0.06[b]	9.4 ± 5.4[a]	0.0095	0.759	0.576
Plasma RBP, *μmol/L*	2.70 ± 0.40[b]	2.45 ± 0.41[c]	2.95 ± 0.53[a]	0.0005	0.746	0.566
EZP, *mmol*	2.54 ± 0.66	1.62 ± 0.35	2.00 ± 0.40	0.042[4]	0.729	0.532
Net body Zn loss, *mg*	—	39 ± 9	—			
Net body Zn gain, *mg*	—	—	108 ± 55			

[1] Values are means ± SD, $n = 5$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] Regression analysis between change in parameter from baseline vs. total change in body Zn.
[3] ND, not determinable (see text for details).
[4] Means did not differ using Tukey's post hoc test.

Total Zn loss and gain. Subjects lost 39 ± 9 mg of Zn during MP2. During MP3, 108 ± 55 mg of Zn was gained, although this was highly variable among subjects, ranging from 30 to 175 mg (**Table 1**).

Plasma and urine Zn concentrations. Plasma Zn concentration fell 74% during depletion ($P < 0.05$).[7] Urine Zn concentration followed a similar pattern, falling by 97% during depletion. Both measures returned to baseline values following Zn repletion (**Fig. 1**). The change in total body Zn was correlated with the change in plasma Zn concentration during Zn depletion and repletion compared to baseline ($P < 0.001$, $r^2 = 0.826$). The relation between 24-h urinary Zn excretion and body Zn change was not as strong ($P < 0.02$, $r^2 = 0.576$; Table 1).

Biochemical measurements. AP activity, measured by SMAC, was significantly lower at the end of MP2 compared to the activity at the end of MP1 (Table 1). RBP concentration also fell significantly as a result of dietary Zn deficiency (Table 1). Both AP activity and RBP concentration returned to levels above baseline following the reintroduction of Zn to the diet. The change in enzyme activity from baseline during MP2 and MP3 correlated ($P < 0.01$, $r^2 = 0.708$) with the change in body Zn levels. The relation between the change in body Zn levels and RBP concentration was weaker ($P < 0.02$, $r^2 = 0.566$).

No significant changes attributable to Zn depletion were observed in the other variables measured by SMAC (data not shown).

Stable isotope studies. The size of the exchangeable Zn pool tended to decline by 36% during Zn depletion ($P = 0.04$), although the means did not differ in Tukey's post hoc test. Plasma Zn flux fell by 51% during the depletion period, and FZA increased 3-fold. Both returned to values close to baseline following Zn repletion (Table 1). Plasma flux and EZE decreased as zinc was lost from the body ($r^2 = 0.766$, $P < 0.001$ and $r^2 = 0.773$, $P < 0.001$, respectively). FZA was inversely associated with the net change in body Zn ($r^2 = 0.755$, $P < 0.002$). The change in the mass of Zn in the EZP correlated with the net change in body Zn ($r^2 = 0.532$, $P < 0.02$).

[7] The values are the means derived from a sample taken from each subject at the stated time point in each metabolic period. In our publication (13), the values used in the model were means from 16 samples taken over 6 d for each subject.

DISCUSSION

In the present study, the acute reduction of dietary Zn intake to about 0.25 mg/d caused a rapid and dramatic fall in plasma Zn concentration, by 20% during the first 11 d of the Zn depletion period. The classic symptoms of Zn deficiency, dermatitis around the mouth and nose and inflammation of the mucous membranes, were observed in subject 2 after only 32 d of consuming the Zn-deficient diet when his plasma Zn

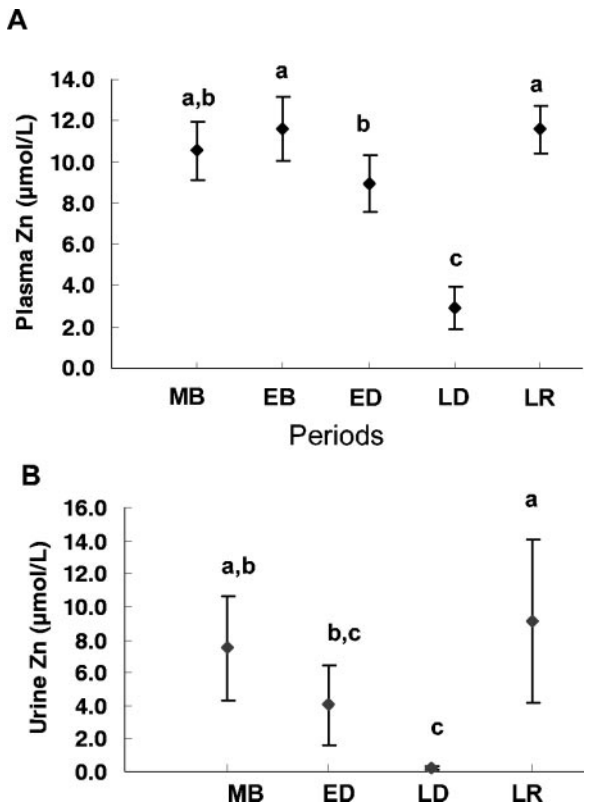

FIGURE 1 Plasma (*A*) and urine (*B*) zinc concentrations in men at the mid- (MB) and end-points (EB) of the baseline period, early (ED) and late (LD) in the depletion period and late in the repletion period (LR). Values are means ± SD, $n = 5$. Means without a common letter differ, $P < 0.05$

concentration fell to 2.6 μmol/L. Symptoms of Zn depletion were first observed in the remaining subjects 35–38 d after the Zn depletion phase was begun. Plasma Zn concentration at that time was 2.6 ± 1.3 μmol/L.

The study described in this paper provided a unique opportunity to examine both conventional biochemical indices of Zn status and the more novel stable isotope techniques for measuring Zn status, as well as to compare these with net Zn loss and gain from the body. The change in plasma Zn concentration correlated best with Zn losses and gains as dietary Zn intake varied ($r^2 = 0.826$). This study confirms that during short-term acute changes in intake, and in the absence of confounding factors, plasma Zn concentration reflects the changes in whole-body Zn status. Plasma Zn concentration appeared to be more sensitive to a loss of whole-body zinc than an increase since the concentration of plasma Zn did not increase above baseline even though the men had a net gain of 90 mg zinc above baseline. EZE also responded to acute changes in zinc intake and correlated well with net loss and gain of zinc from the body. This parameter was identified as a site of homeostatic regulation of zinc metabolism (12) in humans and is conserved when dietary zinc intake is marginal (13). EZE is derived from endogenous zinc secretion, some of which is reabsorbed and the remainder of which is excreted in the stool; therefore, the usefulness of EZE as an indicator of status would be confounded by malabsorption or acute diarrhea.

This study does not support the hypothesis that EZP is a good measure of Zn status following acute changes in dietary Zn intake. During the depletion period, the correlation between net body Zn loss and loss of Zn from the EZP was strong ($r^2 = 0.766$). However, although plasma Zn concentration had returned to baseline values by the end of the repletion period and the men had replaced their losses plus an additional 70 mg, the EZP was about 20% below baseline values. Due to the variability in the response of the individuals, the difference was not significant. At the end of the repletion period, all except 1 of the men had EZP masses lower than that of baseline, varying from 52 to 96% of the baseline value. It is clear, therefore, that Zn kinetics respond rapidly to acute Zn depletion, but a longer period of repletion is required for Zn kinetics to return to baseline values.

From the correlation analysis, FZA reflected net body Zn loss or gain. However, this was primarily due to the data reaching 2 different thresholds. A FZA value of 1 was determined for all 5 subjects during Zn depletion because the model suggested that all of the dietary Zn entering the small intestine was transferred to the plasma, with none entering the lower bowel.

Previous studies in humans investigating the relation between RBP and plasma Zn concentration yielded conflicting results because of the difficulties in controlling for confounding factors such as multiple nutrient deficiencies (5). Similarly, previous reports of the response of AP activity to experimental zinc deficiency in humans were inconsistent (14,15). In the present study, AP activity and RBP concentration clearly responded to changes in dietary Zn intake, falling significantly in response to zinc depletion and returning to values higher than baseline after repletion.

In conclusion, our study confirms that plasma Zn concentration is a valid indicator of whole-body Zn status in the absence of confounding factors, such as infection or stress. Changes in EZP mass correlated well with acute Zn loss from the body, but not with acute Zn gain. Further research is needed to determine how EZP and plasma Zn flux respond to more moderate changes in whole-body zinc, to stress, and to other conditions where plasma Zn concentration is known to be unreliable.

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